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# Correlation between Elevated Bradykinin Concentrations and Death by COVID-19

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## ABSTRACT

**OBJECTIVE:** To investigate BK pathway dysregulation among and between COVID-19 survivors and the deceased.
**METHODOLOGY:** This case-control study was performed between 2020 and 2022 in Imam Hasan Hospital, Bojnurd, Iran. SARS-CoV-2 infected patients, comprising 40 deceased and 15 surviving patients, were recruited according to specific inclusion and exclusion criteria. A blood sample was taken from subjects during the disease. Blood BK levels in subjects (the groups of patients (55) and control (15)) were measured by the ELISA technique. All patients were selected from individuals over 18 years old with real-time PCR-proven SARS-CoV-2 infection. Also, the studied patients did not have metabolic syndrome (blood pressure, abdominal obesity, diabetes, cardiovascular disease). SPSS version 26 was used to compare the means.
**RESULTS:** The blood serum BK level was significantly related to the outcome of COVID-19 disease ($P$=0.006) using a multiple logistic regression test. A week before death, a significant increase in the blood BK levels among deceased patients compared to survivors was seen ($p$=0.0001). The probability of death in patients with SARS-CoV-2 infection linearly increased by 4% (OR = 1.04) for each pg/ml increase in the BK level.
**CONCLUSION:** There is a close relationship between the rise in BK concentration during a COVID-19 infection and the disease outcome.

**KEYWORDS:** Bradykinin, BK, Coronavirus Disease 2019, COVID-19, Severe Acute Respiratory Syndrome coronavirus 2, SARS-CoV-2, inflammatory protein,

## INTRODUCTION

Bradykinin (BK), a peptide in the kinin system, is functionally involved in vascular and renal regulation processes[1]. BK induces mitogenesis and vascular permeability and produces nitric oxide (NO) as an inflammatory mediator in the vascular system and tumor tissues[2].

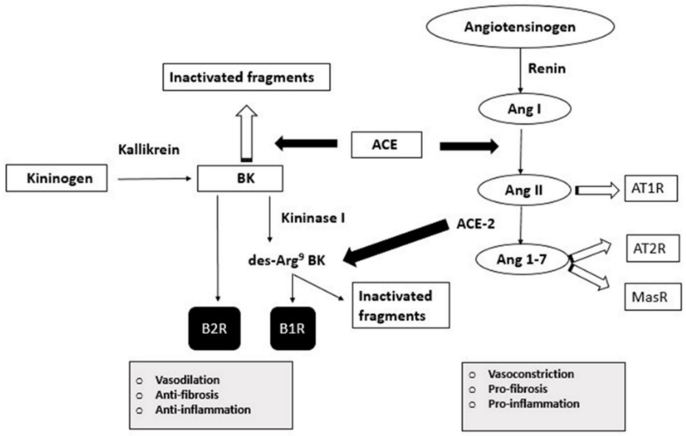
The importance of BK in the clinical outcome of several diseases was reported in past years. The kinin system involves several critical conditions and biological activities, including respiratory allergic reactions, septic shock, and hypertension[3]. The kinin system includes a kininogen substrate that produces BK through kallikrein's enzymatic action, which enhances arterial permeability and vasodilatation[3]. On the other hand, there is a relationship between the kinin and the Renin-Angiotensin system (RAS)[4] (**Figure I**). The kinin and the RAS systems are controlled through the angiotensin-converting enzyme (ACE) that generates Ang II and degrades BK to inactive fragments. BK reduces blood pressure by balancing Ang II under normal conditions[5].

Furthermore, the synergy between mitogen-activated protein kinase (MAPK) and BK confirms the mitogenic role of BK during the growth and proliferation of vascular tissue in smooth muscles after vascular injuries[6]. Due to BK-mediated signalling, inflammatory cytokine upregulation (IL-6, IL-1β, IL-8, and IL-2) is related to BK in many clinical conditions[7] (**Figure I**). This highlights BK as a potential marker for inflammatory disease progression and as a potential therapeutic target. Several papers have focused on increased levels of BK in Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) infection[7-8]. Cell penetration of the SARS-CoV-2 virus is facilitated by the interaction of the spike (S) protein and Angiotensin-Converting Enzyme 2 (ACE-2). Therefore, this internalization can reduce the activity of ACE2 and enhance the concentration of the BK metabolite des-Arg9 BK and its signaling via B1R (**Figure I**). The lung tissue-resident immune cells among SARS-CoV-2 infected patients generate BK by kallikrein activation[9].

SARS-CoV-2 is an exceedingly transmissible virus reported first as the causative agent of Coronavirus Disease 2019 (COVID-19) from Wuhan, China[10,11], and caused significant morbidity and mortality, which it still does to date[12]. The current study aimed to investigate BK pathway dysregulation among and between COVID-19 survivors and the deceased.

**Figure I**. A schematic illustration of ACE regulation mechanism on RAS system and BK. The enzyme ACE can alter Ang I and BK to Ang II and inactive fragments, respectively. Ang 1–7, Ang II, and des-Arg$^9$ BK mediate their effects by Mas, AT1, AT2, B1R, and B2R receptors.

**Figure I:** Schematic illustration of ACE regulation mechanism on RAS system and BK



## METHODOLOGY

This case-control study was performed between 2020 and 2022 in Bojnurd, Iran. Among the patients admitted to Imam Hasan Hospital, 55 were verified patients infected by SARS-CoV-2 and were enrolled in the study to determine their serum levels of BK. A group of healthy blood donors (n=15) were included as the control group.
Patients were aligned into two groups: COVID-19 deceased (n=40) and COVID-19 rehabilitated patients (n=15).
Patients ≥ 18 years old with Real-Time PCR-proven SARS-CoV-2 infection, respiratory rate of 30-40 intakes per minute, oxygen saturation <93% and diagnosis of a pulmonary infiltrate on their chest computerized tomography (CT) scan by a pulmonologist and an infectious disease specialist were included in the study. All study subjects were diagnosed as not having metabolic syndrome (hypertension, abdominal obesity, diabetes, cardiovascular disease).

***Sample collection and BK level measurement:***

Fresh leftover serum (1 ml) from blood samples collected for routine biochemical laboratory tests was stored in a sterile collection tube and kept at minus 30 degrees for future investigation. All blood samples were taken during the disease. Sera collected from healthy blood donors were used to establish a control group.
All serum samples were subjected to enzyme link immunosorbent assay (ELISA) in duplicate to determine serum levels of BK (RayBio® Human/Mouse/Rat Bradykinin ELISA Kit, Atlanta, The U.S.) according to manufacturer's instructions.

***Statistical analysis:***

The independent sample t-test was used to compare the means of BK concentrations among the study groups. A stepwise multivariate regression analysis investigated the relationship between bradykinin levels and disease outcomes among COVID-19 patients. This was done using SPSS software (version 26). Univariate logistic regression analysis examined the relationship between each variable and the designed dependent variable. All variables with a P value ≤ 0.2 were selected for modeling to increase the testing sensitivity. A backward multiple logistic regression test at a significance level of 0.05 was implemented to confirm the estimated relation. The BK level (in pg/ml), sex (female as reference), age of the patient (in years), and hospitalization period (in days) were included as independent variables in the equation.

## RESULTS

***Demographic data of patients:***

Among the 55 participating patients, 58.2% (n=32) were female and 41.8% (n=23) were male. The mean age of patients was 62 (range 24-88) years; among them, 60% (n=33) were above 60.

The group of deceased patients had a mean age of 64.4 years; rehabilitated patients were 58 years on average. The mean age of the control healthy group was 36.2 (19-56) years old, comprising 53.4% females and 46.6% males (**Table I**). The mean age of the control group was lower than that of the patient group, as Iranian regulations for blood donation recommend a maximum donor age of 60 years (18 - 60 years). The mean number of days of hospitalization among deceased and rehabilitated patients was 7.5 and 9.1 days, respectively.

BK levels in blood samples from deceased and age-matched patients who recovered were measured. The mean BK level among patients was 42.35 pg/ml, meaningfully higher than that of the control group ($p$=0.0001, 7.5 pg/ml). Surprisingly, there was a statistically significant difference in BK levels between deceased patients ($p$=0.0001, 49.3 pg/ml) in comparison to rehabilitated patients (23.6 pg/ml) (**Table I**).

The univariate logistic regression test result showed a significant relationship between the blood serum BK level with age ($P$=0.19) and the outcome of COVID-19 disease ($P$=0.006) at the level of 0.2. No statistically significant relation between the BK level with sex ($P$=0.76) or hospitalization period ($P$=0.334) was detected.

According to the multiple logistic regression test, elevated blood serum BK level was significantly related to poorer outcomes of COVID-19 disease ($P$=0.006) at the level of 0.05. For a one-unit (pg/ml) increase in the BK level in blood serum, the probability of death in patients with SARS-CoV-2 infection increased by 4%. (OR = 1.04, $P$ = 0.006) (**Table II**).

**Table I: patient data and measured bradykinin level**

| cases No. | | Gender | | | Measured bradykinin level (pg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Male No. (%) | Female | Age mean ( range) | mean range | *p* | mean range | *p* |
| patients n= 55 | rehabilitated n=15 | 7(46.6) | 8(53.4) | 58(34-86) | 42.35 (7-98) | 0.0001 t=8.968 | 23.6 (7-78) | 0.0001 t=3.815 |
| | dead n=40 | 16(40) | 24(60) | 64.4(24-88) | | | 49.4 (9-98) | |
| control n=15 | | 7(46.6) | 8(53.4) | 36.5(19-56) | 7.5 (3-13) | | | |

*p*= independent sample T-test

**Table II: Results of the univariate and multiple logistic regression analysis**

| Bradykinin | univariate logistic regression | | multiple logistic regression | |
|---|---|---|---|---|
| | OR | *P* | OR | *P* |
| Bradykinin pg/ml | 1.04 | **0.006** | 1.04 | **0.006** |
| Age Median 62 years | 1.02 | **0.19** | 1.02 | 0.25 |
| Sex Female=reference | 0.65 | 0.76 | - | - |
| Hospitalization period Mean=7.5 days | 0.95 | 0.334 | - | - |

Univariate logistic regression test ($P \leq 0.2$ significant), Multiple logistic regression ($P \leq 0.05$ significant), Bold text: Significant

## DISCUSSION

In the present study, the level of BK in the serum of patients with COVID-19 was measured using ELISA. The level of BK in the sera obtained from patients with COVID-19 (42.35 pg/ml) was significantly higher than for healthy individuals (7.5 pg/ml, $P$=0.001). The measured BK level among deceased COVID-19 patients was statistically higher than for rehabilitated COVID-19 patients (49.4 pg/ml and 23.6 pg/ml, $P$=0.0001). The study results revealed a 4% increase in death probability for each unit (pg/ml) increase in the level of BK in the patient's sera.

The increase in the level of BK in sera collected from COVID-19 patients is related to reducing the amount of ACE and ACE2 enzymes. An eight-fold reduced ACE gene expression was already reported among COVID-19 patients[13]. Lower serum ACE levels have already been reported with delayed virus elimination, hyperinflammatory conditions, and impaired host antiviral immune responses[14]. The resulting downregulation of ACE and ACE2 enzymes enhances the activity of BK[7] (**Figure I**). Receptors play a vital role in initiating the action of BK and the intracellular response. BK receptors are cell surface receptors coupled with G-proteins, including the bradykinin 1 receptor (B1R) and the bradykinin 2 receptor (B2R). BK and des-Arg$^9$-BK primarily exert their effects through these receptors, with BK serving as a ligand for B2R, while des-Arg$^9$-BK acts as the primary agonist for B1R[13]. ACE2 is expressed on the surface of respiratory tracts, which causes the breakdown of des-Arg$^9$-BK and prevents its binding to B1R. If ACE 2 function decreases in the lung, the level of des-Arg$^9$-BK increases, which activates B1R and causes the release of inflammatory cytokines and severe damage. While B2R is consistently expressed in various tissues, such as endothelial cells and smooth muscle cells, B1R is a receptor that can be stimulated, and cytokines heighten its expression during infections, immunopathology, and proinflammatory circumstances. Activation of B1R and B2R by their respective ligands increases vascular permeability and neutrophil recruitment, which causes inflammation, pain, and fever, as seen in COVID-19 patients[14].

Other researchers already reported ACE inhibitors as the lead cause of olfactory dysfunctions, dry cough, and taste[15-16]. ACE inhibitor drugs are given to COVID-19 patients to compensate for lowered blood pressure, but there is a concern that those may increase BK and ACE2 concentrations. ACE inhibitors compete with BK for ACE binding sites, thereby leading to a decrease in the degradation of BK and an escalation in the quantity of active BK present within the circulation and tissues. Consequently, this gives rise to angioedema and coughing in patients who are administered these medications[17].

The increase of ACE2, which is the receptor of SARS-CoV-2, works as a double-bladed sword. It functions as an anti-inflammatory agent and a virus attractant[16]. Moreover, enhancing BK, des-Arg9-BK, and Ang-II levels can induce acute respiratory distress syndrome (ARDS) in SARS-CoV-2 infection[18]. Thus, a tight relationship between inflammation and acceleration of life-threatening complications in SARS-CoV-2 infection by raised BK level is predicted.

## CONCLUSION

The current study demonstrates elevated levels of blood BK in COVID-19 patients, thereby suggesting that BK is a novel and highly relevant inflammation-associated protein in SARS-CoV-2 infection. Our finding is consistent with the more recent hypotheses as it shows that increased blood BK levels could worsen the outcome of SARS-CoV-2 infection. One notable paper is "Bradykinin and the pathophysiology of COVID-19: A new perspective" by Garvin et al., published in the journal Lancet Respiratory Medicine in 2020. This paper discusses the role of bradykinin in the pathophysiology of COVID-19 and proposes a new perspective on how the virus may affect the body. Also, increased B1R expression level in liver tissues of patients with COVID-19 is associated with acute liver dysfunction caused by SARS-CoV-2 virus infection in the pathogenesis of COVID-19.
The main limitation of our study is the small number of patients enrolled; we strongly recommend experimental investigation with a large sample size for studying BK levels during SARS-CoV-2 infection. More studies will increase our knowledge to understand if interference with BK signaling can be considered a therapeutic target for overcoming SARS-CoV-2 infection.

**Ethical Statement:** North Khorasan University of Medical Sciences Iran, ERC Letter No. IR.NKUMS.REC.1400.006.
**Consent to participate:** Informed consent was acquired from patients or their legal guardians.
**Conflict of Interest:** No conflicts of interest.
**Financial Disclosure / Grant Approval:** No funding agency was involved in this research.
**Data Sharing Statement:** The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

## AUTHOR CONTRIBUTION

Fani M: Conception and design, material preparation, laboratory works, and analysis
Ghasemzadeh-Moghaddam H: Conception and design, material preparation, writing the first draft of the manuscript, and analysis
Belkum A: Scientific advice
Shoraka HR: Statistical analysis
Azimian A: Laboratory works
Hosseini Z: Sample collection

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